

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-13 – Canceled.

14. (Withdrawn) A method for constitutive and/or inducible gene knock down in a vertebrate, or in a tissue culture or cells of a cell culture derived from a vertebrate, which method comprises stably integrating an expression vector comprising a short hairpin RNA construct under control of a ubiquitous promoter into the genome of the vertebrate, of the tissue culture or of the cells of the cell culture.

15. (Withdrawn) The method of claim 14, wherein the expression vector is suitable for stable integration into the genome of a vertebrate, or into the genome of the tissue culture or of cells of the cell culture.

16. (Withdrawn) The method of claim 14, wherein the expression vector contains homologous sequences suitable for integration at a defined genomic locus through homologous recombination in the genome of the vertebrate, in the genome of the tissue culture or in the genome of the cells of the cell culture.

17. (Withdrawn) The method of claim 16, wherein the cells of the cell culture are embryonic cells.

18. (Withdrawn) The method of claim 16, wherein the homologous sequences are suitable for integration at a polymerase II dependent locus in the genome of the vertebrate, in the genome of the tissue culture or in the genome of the cells of the cell culture.

19. (Withdrawn) The method of claim 14, wherein the expression vector further contains functional sequences selected from the group consisting of splice acceptor sequences, polyadenylation sites and selectable marker sequences.

20. (Withdrawn) The method of claim 18, wherein the polymerase II dependent locus is selected from the group consisting of a Rosa26, collagen, RNA polymerase, actin and HPRT locus.

21. (Withdrawn) The method of claim 14, wherein the ubiquitous promoter is selected from the group of promoters consisting of polymerase I, II and III dependent promoters.

22. (Withdrawn) The method of claim 21, wherein the ubiquitous promoter is selected from the group consisting of a polymerase II or III dependent promoter.

23. (Withdrawn) The method of claim 21, wherein the ubiquitous promoter is selected from the group consisting of a CMV promoter, a CAGGS promoter, a snRNA promoter, a RNase P RNA promoter, a tRNA promoter, a 7SL RNA promoter and a 5 S rRNA promoter.

24. (Withdrawn) The method of claim 23, wherein the S nRNA promoter is a U6 promoter.

25. (Withdrawn) The method of claim 23, wherein the RNase P RNA promoter is a H1 promoter.

26. (Withdrawn) The method of claim 14, wherein the ubiquitous promoter is a constitutive promoter.

27. (Withdrawn) The method of claim 14, wherein the ubiquitous promoter is an inducible promoter.

28. (Withdrawn) The method of claim 27, wherein the inducible promoter contains an operator sequence selected from the group consisting of tet, Gal4 and lac.

29. (Withdrawn) The method of claim 14, wherein said vertebrate is a non-human vertebrate.

30. (Withdrawn) The method of claim 29, wherein the non-human vertebrate is selected from the group of vertebrates consisting of mouse and fish.

31. (Withdrawn) The method of claim 14, wherein the expression vector is a Pol III dependent promoter driven shRNA construct suitable to be integrated into a ubiquitously active Pol II dependent locus;

32. (Withdrawn) The method of claim 31, wherein the PolIII dependent promoter is selected from a constitutive H1 promoter and a constitutive U6 promoter.

33. (Withdrawn) The method of claim 14, wherein the expression vector is a Pol III dependent promoter driven shRNA construct suitable to be integrated into a ubiquitously active Pol II dependent locus.

34. (Withdrawn) The method of claim 33, wherein the Pol III dependent promoter is selected from an inducible U6 promoter and an inducible H1 promoter.

35. (Withdrawn) The method of claim 14, wherein the expression vector is a Pol II dependent promoter driven shRNA construct suitable to be integrated into a ubiquitously active Pol II dependent locus.

36. (Withdrawn) The method of claim 35, wherein the Pol III dependent promoter is a inducible CMV promoter.

37. (Withdrawn) The method of claim 14, wherein the shRNA comprises

(I) at least one DNA segment A-B-C wherein

A is a 15 to 35 bp DNA sequence with at least 95% complementarity to the gene to be knocked down;

B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hair pin molecule, and

C is a 15 to 35 bp DNA sequence with at least 85% complementarity to the sequence A, and

(II) a stop and or polyadenylation.

38. (Withdrawn) The method of claim 37, wherein A is a 19 to 29 bp sequence with 100% complementarity to the gene to be knocked down.

39. (Withdrawn) The method of claim 14, wherein the expression vector is integrated at a polymerase dependent locus of the living organism, tissue culture or cell culture.

40. (Withdrawn) The method of claim 14, wherein the method for constitutive and/or inducible gene knock down in a vertebrate comprises integrating the expression vector into ES cells of the vertebrate.

41.-44. (Canceled)

45. (Currently Amended) A mouse having an expression vector stably integrated by site specific integration at a polymerase II dependent locus of the mouse, said expression vector comprising a short hairpin RNA construct under control of a ubiquitous polymerase III-dependent promoter and sequences suitable for targeted integration at the polymerase II dependent locus, wherein expression of said short hairpin RNA construct results in repression of expression of a gene targeted by said short hairpin RNA construct in said mouse.

46. (Canceled)

47. (Currently Amended) A tissue or cell culture obtained from a mouse, cells of said tissue or cell culture having an expression vector stably integrated by site specific integration at a polymerase II dependent locus of the cells, said expression vector comprising a short hairpin RNA construct under control of a ~~ubiquitous~~polymerase III-dependent promoter and sequences suitable for targeted integration at the polymerase II dependent locus, wherein expression of said short hairpin RNA construct results in repression of expression of a gene targeted by said short hairpin RNA construct in said ~~mousetissue or cell culture~~.

48. (Currently Amended) An expression vector comprising a short hairpin RNA construct under control of a ~~ubiquitous~~polymcrase III-dependent promoter and sequences suitable for targeted integration at a polymerase II dependent locus of a mouse.